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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s)	:	Dieter MANSTEIN et al.
Serial No.	:	10/542,390
Filed	:	July 13, 2005
For	:	METHOD AND APPARATUS FOR DERMATOLOGICAL TREATMENT AND FRACTIONAL SKIN RESURFACING
Examiner	:	David Shay
Art Unit	:	3769
Confirmation No.	:	3451

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR § 1.131

I, Dieter Manstein, M.D., declare as follows:

1. I am a co-inventor of the above-identified application, and inventor of independent claims 63, 90 and 113, as provided in the Amendment and Response to Final Office Action submitted herewith. I am familiar with the contents of the patent application and the prosecution undertaken therein as of the date of the present declaration.

2. I am also a Clinical Fellow in the Wellman Center for Phototherapy at the Massachusetts General Hospital in Boston, Massachusetts, and an Instructor in Dermatology at Harvard Medical School in Boston, Massachusetts.

3. Exhibit A contains a copy of an Invention Disclosure Form submitted to the Massachusetts General Hospital before October 22, 2002. Exhibit B is a research grant application that I prepared for submission to the American Society for Laser Medicine and Surgery (ASLMS) before October 22, 2002. Exhibit C is a copy of an image of a pattern of small damaged regions of skin tissue, including both epidermal and dermal damage, that was generated in a

test subject prior to October 22, 2002. In particular, these Exhibits A, B and C were prepared by me, under my direct supervision, and/or in collaboration with the other co-inventor of the above-referenced patent application. All dates on the attached Exhibits A, B and C have been redacted unless otherwise stated herein by reference to specific dates.

4. Prior to October 22, 2002, I conceived the invention recited in claims 63, 90 and 113 in the above-identified application (provided in the Amendment and Response to Final Office Action submitted herewith), as evidenced by the attached Exhibits A, B and C.

5. The exemplary data, apparatus and method descriptions provided in Exhibits A, B and C disclose certain aspects of the fractional resurfacing invention for skin tissue using lasers that is disclosed and claimed in the present patent application, whereby certain regions of damage are formed from the skin surface through the epidermis and into the dermis, and whereas such regions are separated by regions of healthy or undamaged tissue to promote rapid healing and desirable aesthetic results such as skin tightening and wrinkle removal.

6. Exhibit A is a copy of an Invention Disclosure Form that describes the general concept and certain exemplary details of the fractional resurfacing invention described and claimed in the present application. The type of tissue damage, including damage to a portion of the dermis, is illustrated in the side-view figure on page 3 of Exhibit A. It was also understood by one of ordinary skill in the art at the time the present application was filed that a skin "resurfacing" procedure produces damage or removal of both epidermal and dermal tissue, in contrast to a "peeling" procedure that would involve only damage or removal of a portion of the epidermal layer.

7. Exhibit B is a copy of a research grant application that describes the general concept and certain exemplary details of the fractional resurfacing invention described and claimed in the present application. Further, analysis of the epidermal and dermal damage to be produced under the proposed research is described on page 8 (section 11) of Exhibit B.

8. Exhibit C is an image showing an exemplary pattern of small regions of damage (e.g., less than 1 mm in size) that were formed from the skin surface through the epidermis and into the dermis (some of which are visible in the image as dark spots to the right of the "M" label).

This exemplary pattern of damage was produced using the exemplary apparatus and method recited at least in claims 63, 90 and 113 (as provided in the Amendment after Final Office Action submitted herewith) prior to October 22, 2002.

9. As evidenced by the Exhibits A, B and C, the exemplary apparatus and method which included every element recited at least in claims 63, 90 and 113 (as provided in the Amendment after Final Office Action submitted herewith) were described by me in detail and physically implemented by me and/or under my direction or control earlier than October 22, 2002, thus reducing the invention recited in such claims 63, 90 and 113 to practice subsequent to the conception thereof and earlier than October 22, 2002.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

11/23/09
Date

Dieter Manstein, M.D.

EXHIBIT A

OTA use only
Disclosure Received: _____
Disclosure No:_____

MGH INVENTION DISCLOSURE FORM
OFFICE OF TECHNOLOGY AFFAIRS

1. **TITLE OF INVENTION**:

Enhanced technique for skin resurfacing providing anesthesia, faster wound healing, treatment of all skin types and various body sites.

2. **INVENTOR(S) NAME, TITLE, LAB, DEPT., ADDRESS AND TEL. EXT.**:

Rox Anderson, Assoc. Prof, Wellman Lab, Dermatology, 6-6148
Dieter Manstein, Research Fellow, Wellman Lab, Dermatology, 6-4893

3. **SOURCE OF FUNDS FOR THE RESEARCH WHICH RESULTED IN THE INVENTION**:

A.		Government Grant - Agency and Grant No.	
B.	x	Private Industry - Name: XXXXXXXXXX	
C.		MGH	
D.		Foundation	
E.		Other- Explain	

EXHIBIT A

4. **SUMMARIZE THE CLAIMED NOVELTY ASSOCIATED WITH THE INVENTION:**

Laser assisted skin resurfacing using CO_2 or Er:YAG laser is a well established procedure for various indications including wrinkle removal, scar improvement, ablation of superficial skin lesions. By using a cooled (metal) grid in direct contact to the skin while applying the laser beam two major advantage can be achieved. Anesthesia of the skin and faster recovery of the epidermis because areas of epidermis are spared (island of saved tissue). The concurrent hypothesis is that the reepithelization and repigmentation is proceeding from the spared tissue of the follicular tissue within the dermis. Applying a mesh to the epidermis can increase the density of this spared epitheliar tissue. It is expected that the time for reepithelization and repigmentation is decreased. Recovery time will be faster. Especially melanocytes are known for their low migrating properties. So decreasing the distance of migration should have a major impact on the repigmentation. Because of having a faster repigmentation (that means shorter down time) this technique might allow resurfacing of all skin types. An additional effect of applying a cooled mask is providing anesthesia. By using deeper penetrating wavelengths this technique can be used for nonablative resurfacing procedures as well.

5. **INVENTION DISCLOSURE**:

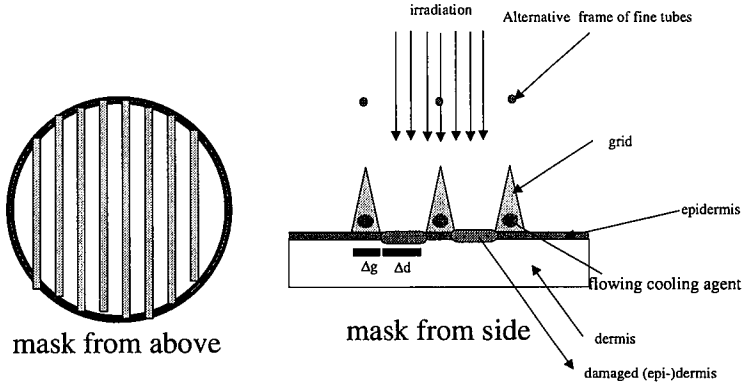
Please attach a description of the invention in sufficient detail, using the outline below to convey a clear understanding of the nature, purpose, operation and the physical, chemical biological or electrical characteristics of the invention. Attach sketches, drawings, photos, diagrams or photos, and any pertinent manuscript which described the invention:

The purpose of the invention is to decrease the time for reepithelization, repigmentation and to decrease pain. The basic idea is to keep areas of the epithelium undamaged (island of saved tissue). These areas serve as a pool of undamaged cells that can promote reepithelization. The current hypothesis is that after resurfacing procedures the reepithelization initiate from the depth of undamaged follicular epithelium. Because current procedures remove the entire epithelium, an important factor for the time of reepithelization is the density of follicles. The vellus hair density of the face (439 hairs/cm^2) is much higher than on the back (85 hairs/cm^2) [Blume et al.: Physiology of the vellus hair follicle: hair growth and sebum excretion. Br J Dermatol 1991; 124(1):21-8]. This is the current explanation for the better and faster reepithelization of the face in comparison to other body areas with lower hair density. CO_2 laser resurfacing of other areas than the face is currently not recommended because of increased side effects [Jimenez G et Spencer JM: Erbium: YAG Laser Resurfacing of the Hands, Arms, and Neck. Dermatol Surg 1999 Nov;25(11):831-835]. The "travel length " of a keratinocyte or melanocyte after complete deepithelization would be up to 480 µm for the face versus 1084 µm for the back. Various publications have demonstrated that keratinocytes can improve wound healing [Arambula H et al.: Frozen Human Epidermal Allogenic Cultures Promote Rapid Healing of Facial Dermabrasion Wounds. Dermatol Surg 1999 Sep:25(9):708-712]; and [Fratianne

EXHIBIT A

R et al.: Keratinocyte allografts accelearate healing of split-thickness donor sites: applications for improved treatment of burns. J Burn Care Rehabil 1993 Mar-Apr;14(2 Pt1):148-54]. By applying a cooled (metal) grid or mesh to the surface some keratinocytes and melanocytes are protected from thermal damage. Beside of the saved cells in the depth of the vellus hair follicle there would be additional cells available for promoting wound healing. Melanocytes are not migrating well and therefore a decreased distance will probably improve the repigmentation process. It can be that the repigmentation process is improved so much that even treatment of all skin types might be possible. The protection of saved epidermal cells is achieved by a combination of shielding the laser radiation and cooling the underlying keratinocytes and melanocytes. The technique of using a cooled contact mask is probably much more effective for that purpose than a scanning laser device. It is assumed that the improved wound healing is caused by the increased pool of surviving epithelial cells and the decreased distance of cell migration. Also resurfacing of areas so far not accessible for CO_2-laser resurfacing like neck, and hands might be become an area that can be successfully resurfaced with this enhanced technique. The design of the mask should consider the density of vellus follicles, mechanical stability of the mask, sufficient cooling capacity of the mask, minimal loss of light. The exact design of the mask will be optimized after clinical trials but the following is a potential design of the mask:

potential design of the mask



There are two different options to cool the metal mask: The heat is removed just by heat diffusion of the metal mask or there is a circulating cooling agent (gas or liquid) circulating through micro channels within the mask (see figure). It is also possible to frame thin tubes (50-200 μm diameter) with a circulating cooling agent.

The diameter of the hole mask should be matched to the size of the ablation pattern of most of the already commercially available laser devices. The inner

diameter of the supporting frame should be therefore in the range between 10 to 20 mm. The diameter of the shielding structures within the mask should be in the range of 50 to 300 μm. The diameter of the apertures of the mask should be in the range of 100- 1000 μm. The ratio of shielding versus opening will probably affect the clinical outcome. Also it might be useful to use a mask with small openings to generate a transition zone at the edge of a resurfaced area. It is expected that the optimum mask will vary for different indications, skin types and body areas. The exact dimensions will be optimized during clinical trials. The surface of the mask should have a minimal absorption at the wavelength used for resurfacing in order to decrease unwanted heating of the mask. Also the design should consider safety aspects for the back reflected laser light in order to not cause laser accidents. Therefore the mask should be mounted to the handpiece of the laser, should be manufactured of a highly scattering (micro structured) surface and should avoid large areas reflecting the laser light to the operator.

As described in a separate disclosure, skin cooling can provide a significant anesthesia during resurfacing procedures. Using a cooled metal mask to achieve cooling can solve various problems related to different ways of contact skin cooling. If a transparent window is used for the procedure contamination and shielding of the window by ablated debris is a major problem. If cooling is achieved by precooling with a cold plate, this plate has to be removed before the laser pulse can be delivered. This implies a more complicated procedure and the time necessary to remove the plate causes a delay in performing the procedure. The use of a cooled mask can avoid these problems because the debris can not be attached to the apertures of the mask and would be ablated by consecutive pulses. No delay is caused by removing the mask before applying the pulse.

The technique of applying a mask in order to selectively spare islands of cells for tissue regeneration might be applicable not only for superficial resurfcing techniques but also for the more popular becoming subsurfacing techniques. Just by changing the wavelength to a deeper penetrating wavelength, in depth damage of small areas can be achieved without damaging an entire layer within the tissue. Also the safety is increased because the pattern of the mask protects certain areas within the skin. This can improve tissue regeneration.

EXHIBIT A

6. PATENT AND LITERATURE SEARCH

Please undertake a patent and literature search relating to this invention. Provide copies of any relevant bibliography or individual documents. Note, you may choose to use the IBM Patent search utility (" http://patent.womplex.ibm.com/ ") or the National Library of Medicine clinical search utility.
No patents of using a mask to enhance laser treatment of skin were found.

7. NON-CONFIDENTIAL DISCLOSURE INFORMATION

Please attach and e-mail a non-confidential summary comprising several paragraphs detailing your invention in a broad non-specific fashion. You should provide sufficient detail to identify the field of your invention but should not divulge the novelty inherent in your invention. This disclosure may be used for marketing purposes by the Office of Technology Affairs.

Non-Confidential Disclosure Information Attached Y _____

8. PUBLICATION, SALE OR USE OF THE INVENTION:

A. Have you described your invention in a publication or presentation ?

YES _____ NO X

1. If YES, give name and date of publication or presentation.

2.. If NO, describe any plans which you may have for publication or presentation in the future.

__scientific publication will be submit in XXXXXXX_____

B. Has your invention been used or offered for sale ? Has it been used on patients at MGH or elsewhere ? When and under what circumstances?

No

EXHIBIT A

9. **COMMERCIAL POSSIBILITIES**: To the extent known, please state whether the invention has significant commercial potential. Is the invention primarily a research tool? Does it appear to have significant commercial potential outside the United States ?

Resurfacing and skin rejuvenation is a multi million dollar market worldwide. A new enhanced technique that would allow treatment of various skin areas, all skin types with decreased pain and reduced down time represents certainly a very significant value. Because this enhanced technique could be used in combination with current distributed laser systems the market acceptance is expected to be high.

Identify any potential licensees which you are aware of or have had contact with.

Palomar Medical Inc, Burlington
Coherent Medical, Palo Alto

10. **HISTORY OF THE INVENTION**:

A. When did you first think of (conceive) the invention?

Date XXXXXXX .

B. When did you first disclose your invention to another person?

Date XXXXXXX To Whom XXXXXXXXX

C. XXXXXXX When was the first written description or drawing of your invention produced? Please attach photocopy of such written description.

Date XXXXXXX .

11. **INTERACTIONS WITH THIRD PARTIES**:

A. Have you or any co-inventor(s) listed in (2) above received equipment, drugs or biological materials from any industrial or academic source for use in the research which gave rise to the invention? If so, please list and attach a copy of any applicable Agreements.

EXHIBIT A

B. Have you or any co-inventor(s) listed in (2) above entered into or signed a confidentiality or secrecy Agreement in exchange for receiving any proprietary information from a third party pertaining to the research which gave rise to the invention? If so, please describe briefly the subject or the confidentiality or secrecy Agreement (s) and attach a copy of each such Agreement.

INVENTOR(S)' SIGNATURE(S): DATE

____R. Rox Anderson_____ XXXXXX_____

____Dieter Manstein_____ XXXXX_____

WITNESS(ES): Disclosed to and understood by me on:

DATE SIGNATURE

_____ _____

_____ _____

Please return completed form to:

David Glass, Ph.D.
Office of Technology Affairs
Massachusetts General Hospital
13th Street, Bldg., 149, Suite 1101
Charlestown, MA 02129
Mail Code: 149-1101

or

Iain D. Miller, Ph.D.
Office of Technology Affairs
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EXHIBIT A

e-mail: glass.david@mgh.harvard.edu

e-mail: miller.iain@mgh.harvard.edu

EXHIBIT B

A. WARD FORD MEMORIAL INSTITUTE GRANT APPLICATION

Organization Name:	**Wellman Laboratories of Photomedicine**
Contact Person:	**Dieter Manstein, MD**
Address:	**50 Blossom Street, BHX 630**
City:	**Boston, MA 02114**
Country:	**USA**
Telephone:	**617 726-4893**
Fax:	**617 724 2075**
Email:	**Dmanstein@partners.org**

The above named agency's governing board has authorized this project:

__X__Yes _____No

....

Signature of Board President **Signature of Executive Director**

....

Typed Name of Board President **Typed Name of Executive Director**

EXHIBIT B

WARD FORD MEMORIAL INSTITUE GRANT APPLIACTION:

1. Total project bud get: $ 47.400
   Amount requested from the ASLMS $ 47.400

2. Anticipated project sta rt date: XXXXXXXX

   Anticipated project end: XXXXXXXX

3. Potential impact of research:

"**Fractional resurfacing**" * is a new concept and technique for improving laser treatment of the skin. We expect fractional resurfacing to provide faster healing, fewer side effects and greater safety in a wide range of skin types compared with conventional laser resurfacing.

* Patent application for the concept of "fractional resurfacing" is pending.

THE PROJECT:

4. Describe the project for which you are seeking funds. Please include specific objectives of the project.

Background and Significance: Laser skin resurfacing using CO_2 or Er:YAG laser is a well established procedure for various indications including wrinkle removal, scar improvement, ablation of superficial skin lesions [Weinstein C. Carbon dioxide laser resurfacing. Long-term follow-up in 2123 patients. Clin Plast Surg 1998 Jan; 25(1):109-30]. However the removal of the entire epidermis and residual thermal damage of dermis, causes significant side effects and is associated with prolonged healing during a "down time" of up to several weeks when the patients are limited in their social activities. [Alster TS, Lupton JR. Complications of laser skin resurfacing. Facial Plast Surg 2000 May; 8(2):163-72]. Based on our current knowledge, reepithelization of the epidermis after resurfacing initiates from stem cells in the hair follicles, which serve as a reservoir of non-damaged epithelial cells. Follicular epithelial stem cells must proliferate, migrate, and form a new basement membrane to cover the skin after conventional laser resurfacing. Because current procedures remove the entire superficial layer of epidermis, an important factor for the time of reepithelization is the density of follicles. The vellus hair density of the face (439 hairs/cm^2) is much higher than on the back (85 hairs/cm^2) [Blume U, Ferracin J et al. Physiology of the vellus hair follicle: hair growth and sebum excretion. Br J Dermatol 1991; 124(1):21-8]. This is a putative explanation for the better and faster reepithelization of the face in comparison to other body areas with lower follicular density. CO_2 laser resurfacing of areas other than the face is currently not recommended because of increased side effects [Jimenez G, Spencer JM. Erbium: YAG Laser Resurfacing of the Hands, Arms, and Neck. Dermatol Surg 1999 Nov; 25(11):831-835]. The "migrating path-length" of a keratinocyte or melanocyte for complete deepithelization would be up to 480 μm for the face versus 1084 μm for the back. Various publications have demonstrated that keratinocytes allografts can improve wound healing [Arambula H, Sierra-Martinez E et al. Frozen human epidermal allogeneic cultures

promote rapid healing of facial dermabrasion wounds. Dermatol Surg 1999 Sep; 25(9):708-712] and [Fratianne R, Papay F et al. Keratinocyte allografts accelearate healing of split-thickness donor sites: applications for improved treatment of burns. J Burn Care Rehabil 1993 Mar-Apr; 14(2 Pt1):148-54]. However, allografts are difficult for both economic and practical reasons after laser skin resurfacing.

Pigmentary changes are a major and long-lasting side effect after laser resurfacing, which limits the technique in darker-skinned patients. Melanocytes along the dermo-epidermal junction are completely removed by conventional skin resurfacing, and are replaced from melanocyte stem cells in hair follicles, similar to the epithelial cells discussed above. Vililigo is an autoimmune skin disease which offers some insight into this process. Only those vitiligo patients who still have pigmented hairs are able to respond to medical or phototherapy, which causes repigmentation. Patients with leukotrichia (white hairs) can be repigmented, however, if epidermal melanocytes are transplanted by autologous grafting. [Malakar S., Dhar S. Treatment of stable and recalcitrant vitiligo by autologous miniature punch grafting: A prospective study of 1000 patients. Dermatology 1999; 198:133-139]. This is also an established technique for repigmentation of leukoderma [Falabella, R. Surgical repigmentation of stable leukoderma by autologous mingrafting. J Dermatol Surg Oncol 1986; 12:172-179]. Grafting is a relatively complicated and time-consuming procedure, but offers the insight that re-pigmentation and possibly pigmentary side-effects, should be improved by intentionally leaving some residual, viable melanocytes after skin resurfacing.

To solve these problems, I propose a new concept and practical method in which millions of nearly-invisible islands of viable epidermis are spared during laser resurfacing. Our approach is to maintain a certain fraction of keratinocytes and melanocytes of the inter-appendageal epidermis undamaged. These undamaged cells can serve as a proliferating cell pool. The hypothesis is that the not damaged fraction of epithelial cells is potentially equivalent to autologous minigrafting. This "seeding" of millions of autologous cells over the resurfaced area is expected to provide faster and enhanced reepithelization and repigmentation. We call this new concept: "**fractional resurfacing**".

Resurfacing of darkly pigmented skin is generally not recommended because of increased risk of transient hyperpigmentation and permanent hypopigmentation. Melanocytes are known for their limited migrating properties. Therefore decreasing the distance of cell migration can potentially enhance not only speed of repigmentation but also the capability for repigmentation. Fractional resurfacing therefore potentially enables resurfacing of <u>all skin types.</u>

Specific Aims:

a) Develop and optimize a resurfacing procedure that selectively spares an array of small areas of epidermis from damage.
b) Demonstrate that these spared areas can be generated in an invisible (to the naked eye) pattern, such that they do not cause a heterogeneous (patchy) appearance of the skin.
c) Perform a pig study to evaluate systematically the effects of pattern and spacing of 'fractional resurfacing' on wound healing, specifically reepithelization and repigmentation.
d) Perform a pilot human study of fractional resurfacing to examine:

EXHIBIT B

- What are the side effects of 'fractional resurfacing' compared to standard resurfacing technique?
- What is the efficacy of 'fractional resurfacing' compared to the standard resurfacing technique?
- Is resurfacing of dark pigmented skin possible with 'fractional resurfacing'?

Material and Methods:
A simple and novel approach will be taken, to preserve small islands of epidermis, using a micro-mesh applied to the skin surface in combination with a standard CO_2 resurfacing laser (Ultrapulse Coherent laser with computer pattern generator handpiece). The function of the micro-mesh is to act as a both, an optical mask, shading the underlying epidermis from laser exposure, and as a thermal sink, cooling the underlying patch of epidermis. The mesh will be made of thermally-conductive, optically-reflective (at 10,600 nm) metal to optimize cooling and minimize absorption of laser radiation by the mesh. The design of the mask depends on various factors and should consider the density of vellus follicles, mechanical stability, sufficient cooling capacity, minimal loss of laser light, pattern size below that easily seen with the naked eye, and biological response of the tissue to damage pattern. These factors have been considered in design of the prototype described below. Performance will be confirmed and optimized in vitro, followed by an animal wound healing study and a pilot human study in vivo. The proposed research is intended not only to test the feasibility of fractional resurfacing, but to answer hypotheses about wound healing after laser resurfacing, and lead to further support via industry or NIH.

potential design of the mask

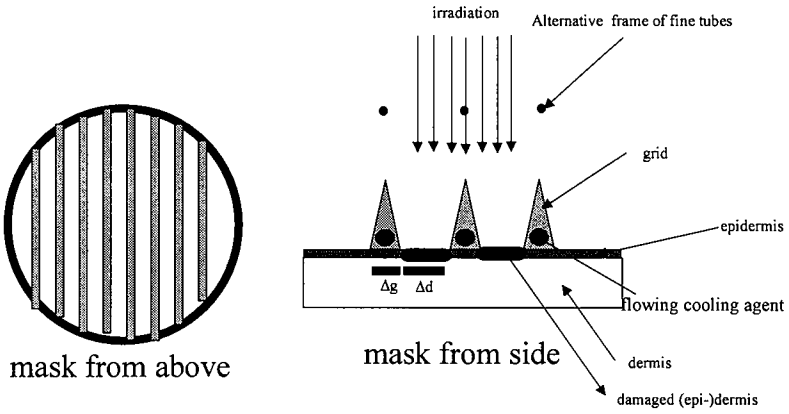

EXHIBIT B

There are several options to cool the mask, which in turn extract heat by conduction from patches of epidermis immediately underlying the mask. A simple approach is to cool the mask just before application to the skin surface. The mass, size and heat capacity of the mask become critical factors in this approach. Alternatively, a circulating cooling agent (gas or liquid) can be used, flowing through channels within the mask (see figure). This approach can potentially give stronger cooling, but is more complicated to construct and operate. It is possible, however to frame thin tubes (50-200 μm diameter, similar or identical to 30-gauge or greater hypodermic needle stock), supplied with a circulating cooling agent of low viscosity.
The diameter of the entire mask should be matched to the size of the ablation pattern of the laser used for resurfacing. In the proposal, the Coherent Ultrapulse laser is selected for several reasons – it provides a range of patterns and pattern sizes, uniform and well-collimated beam optics, very rapid pattern scan time, independent control of pulse energy, and a short, fixed pulsewidth which limits lateral thermal diffusion under the mask. The inner diameter of the mask's supporting frame will be therefore in the range between 10 to 20 mm. The diameter of the shielding structures within the mask will be in the range of 50 to 300 μm. The diameter of the apertures of the mask will be in the range of 100- 1000 μm. The ratio of shielding versus opening will probably affect clinical outcome. Also it might be useful to use a mask with small openings to generate a transition zone at the edge of a resurfaced area. Although the optimum mask may vary for different indications, skin types and body areas, this proposal focuses on designs for facial resurfacing.

The surface of the mask will have minimal absorption at the wavelength used for resurfacing in order to decrease unwanted heating of the mask. Also the final design should consider safety aspects for the back reflected laser light in order not to cause laser hazards. Therefore the mask will be mounted to the handpiece of the laser and made of a highly far-infrared reflecting and scattering (micro-structured) surface such as brushed copper or gold. It is also planned to test various commercially available metal (e.g. copper) masks in the in-vitro study. These masks, even if built for other purposes like filtering, holders, electron-microscopy or other applications might be able to generate the desired ablation and damage pattern.

In vitro-testing:
In order to optimize the design of a mask for 'fractional resurfacing' we will perform initially in-vitro testing on post mortem human skin. This initial in-vitro testing is also necessary to investigate the impact of the design of the mask (material, pattern, thickness, cooling), operational factors (timing of the cooling, contact pressure, effects of multiple passes) and laser parameters (fluence, repetition frequency, overlap of pulses) on the thermal damage pattern of the epidermis. We will then select the most promising combination of mask design, laser parameters and operational procedure for further testing on a pig skin model. On the basis of physical and thermal models for the mask and underlying epidermis, we expect to find that the mask will spare epidermis under itself, down to a mask size of 0.2 mm or less. We anticipate that the most practical diameter of spared patches of epidermis, will be approximately 0.1 mm (i.e., about equal to the thickness of the epidermis). Our concept is that the most promising combination is probably a damage pattern between the islands of epidermal sparing, that is close to that achieved by standard resurfacing techniques. However

we would like to maintain small areas of undamaged keratinocytes and melanocytes within the resurfaced areas. These areas should not be macroscopically visible and should probably have a distance between themselves smaller than the distance between hair follicles. We will use a post mortem human skin model for the initial evaluation of the histologic damage pattern after resurfacing with the mask for various laser parameters. The thermal damage pattern will be monitored by histochemical NitroBlueTetrazoliumChloride (NBTC) evaluation technique. This technique can demonstrate thermal damage in tissue after laser exposure [Hukki J, Lipasti J et al. Lactate dehydrogenase in laser incisions: a comparative analysis of skin wounds made with steel scalpel, electrocautery, superpulse-continuous wave carbon dioxide lasers, and contact Nd:YAG laser. Lasers Surg Med 1988; 8:108-118.].

Animal study:
After completion of the in-vitro testing and assessment of the most promising mask design and parameters, an animal study will be conducted. A pig skin model can be used to evaluate the effects of resurfacing on wound healing after resurfacing [Ross EV, Barnette DJ et al. Effects of overlap and pass number in CO_2 laser skin resurfacing: a study of residual thermal damage, cell death, and wound healing. Lasers Surg Med 1999: 24:103-112.]. Because we are specifically interested in the reepithelization and repigmentation we use a black Yucatan Micropig as a model.
A prospective, open-label, study design will be used. Up to 3 black pigmented, about 6 months old Yucatan Micropigs will be used. Conventional resurfacing with Ultrapulse CO_2-Laser (Coherent) will be compared with Fractional Resurfacing at mapped test sites using the same laser and a sterile mask design based on the in-vitro study analysis. The mask may be as simple as a modified commercially-available copper wire micro-mesh, or may be manufactured specifically for the study. Specifically we would like to assess the repigmentation for different patterns. The repigmentation rate for different distances between the spared areas and for different resurfacing fractions (ablated vs spared ratio) will be assessed for given laser parameters. Pre-treatment assessment will include mapping, film and digital photography under standardized lighting conditions (parallel and crossed polarizations). The animals will be anesthetized for the procedure and every follow-up. At day 1, 7, 14, 21 and 42 clinical and digital photography and biopsies will be performed. These biopsies will be processed for H&E, Fontana-Masson stain and dopa-reaction to assess wound healing, reepithelization and repigmentation. At day 1 the biopsies will be additionally processed for NBTC to assess the thermal damage.

After completion of the animal study a human pilot study will be conducted. The main goal of this clinical trial is to compare side effects and efficacy of fractional resurfacing versus the conventional resurfacing technique in a side by side comparison.

Planned clinical study design: A prospective, open-label, single arm comparative pilot study design will be used. Up to 10 adult volunteers with facial rhytids and/or acne scarring, of Fitzpatrick skin types I-IV will be enrolled. If the results of the pig study demonstrate enhanced repigmentation for fractional resurfacing we consider to enroll all skin types. Conventional resurfacing with Ultrapulse CO_2-Laser (Coherent) will be compared with Fractional Resurfacing using the same laser and a sterile mask design based on the in-vitro study analysis. The mask used for the study will be selected according to the most promising

results of the previous in-vitro and animal testing. Pre-treatment assessment will include film and digital photography under standardized lighting conditions (parallel and crossed polarizations), confocal microscopy of mapped areas, and clinical assessment by the investigator. Based on a coin flip, one side of the face will be treated conventionally, and one side using Fractional Resurfacing. Follow up at day 1, day 3, 1 week, 2 weeks, 1 month and 3 months will include photography under the same standard conditions, confocal microscopy of mapped areas, clinical assessment by investigator and patient questionnaire. The confocal microscopy will be performed in vivo, using a Lucid model 1000 infrared scanning laser microscope. This device is approved by FDA for human use, and provides non-invasive imaging of the entire epidermis and upper dermis without the need for a biopsy. However upon subject's agreement optional 2mm biopsies will be obtained.

5. If clinical research, has it been approved by your I.R.B.?

The approval of the I.R.B. is pending. We do not anticipate significant barriers to IRB approval, based on previous clinical studies experience.

6. If you are seeking a continuation for a project previously funded by the A. Ward Ford Memorial Institute, please describe the progress made on the project in the prior year's funding.

N.A.

7. Why is the project appropriate for ASMLS funding?

Part of the mission of ASMLS is to support and develop new laser devices and procedures. This project is directly aimed at discovering and developing a new laser assisted procedure in Dermatology beneficial for many patients. Therefore we believe that the ASLMS is the most appropriate institution to apply for funding of this project.

8. Who is the project intended to serve?

The goal of the project is to provide a resurfacing procedure with less side effects. This would be beneficial for all patients seeking for resurfacing procedures for a variety of indications (like hypotrophic acne scarring, rhytidectomy, skin rejuvenation). However, our work will especially benefit dark-skinned patients, who presently suffer the greatest side-effects from laser resurfacing. This is could facilitate the access of ethnic groups to resurfacing procedures. Also this project is expected to provide a better understanding of the wound healing after laser resurfacing.

9. What benefit will be derived form this project?

We expect to derive the following benefits from the project:

- Development of a resurfacing procedure with less side effects and downtime

EXHIBIT B

- Development of a resurfacing procedure for ethnic and pigmented skin types and therefore access of ethnic skin types to resurfacing procedures.
- Enhanced understanding of the new concept of "fractional resurfacing" and wound healing after laser resurfacing

10. Outline the components (activities, strategies, equipment allocations, etc.) of the project.

Plan:

- Design and realization of various mask prototypes according to theoretic considerations
- In-vitro testing on post mortem human skin model for optimization of mask and operational procedure
- Animal pig study to evaluate systematically the effect of fractional resurfacing on wound healing
- Clinical pilot study for comparison of efficacy and side effects of fractional resurfacing versus conventional resurfacing

We plan to perform the entire project within our Lab. We do have personnel experienced in design and realization of medical prototypes. Our Laboratory does have a histopathological laboratory and all the equipment and personnel to perform the in-vitro testing, animal study and the pilot clinical trial.

11. How will the project be evaluated? Please be specific.

For the in-vitro testing we will use a post mortem human skin model for the evaluation of the histological damage pattern after resurfacing with various masks for various laser parameters. The thermal damage pattern will be assessed by histology (NBTC stain). In order to optimize the mask design we will evaluate specifically the following for various masks and laser procedures: ratio of damaged vs no-damaged epidermis, size of non-damaged epidermal areas, correlation of shading from the mask with non-damaged epidermis, dermal damage depth and shape (edge effects).

After completion of the in-vitro testing and assessment of the most promising mask design and parameters, a pig study will be conducted. The wound healing, repigmentation and reepithelization will be systematically investigated for fractional resurfacing versus conventional resurfacing on a black Yucatan Micropig model. Specifically we would like to assess the effects on wound healing for different patterns. The repigmentation rate for different distances between the spared areas and for different resurfacing fractions (ablated vs spared ratio) will be assessed for given laser parameters by digital imaging and histology.

Clinical pilot testing: After completion of the animal study the effects of fractional vs standard resurfacing will be evaluated at a prospective, clinical pilot study. Up to 10 subjects of eventually all skin types (depending on the results of the animal testing) will be enrolled. The follow-up intervals are 1 day, 3 days, 1 weeks, 2 weeks, 1 month and 3 months. Clinical pictures will be assessed concerning side effects and efficacy graded by a blinded expert panel. Digital macroscopic pictures will be analyzed concerning the rate of reepithelization,

erythema, hypopigmentation, hyperpigmentation and repigmentation. Confocal microscopy of mapped areas will be performed to monitor reepethelization and repigmentation. This technique allows in-vivo to measure quantitatively the thickness of the epidermis and melanocytes density. Biopsies will be obtained upon subject's agreement. A questionnaire of the subject will assess subject's satisfaction and subjective side effects.

How do you intend to publicize your project and include ASLMS in your publicity?

We plan to submit the results of this study to Lasers in Surgery and Medicine.

THE APPLICANT:

13. Explain your organization's special qualifications to undertake this project.

The Wellman Laboratories of Photomedicine has a long and recognized history for the development of new laser assisted procedures in various disciplines. All of the equipment necessary, including the laser and confocal microscope devices, are available here. Massachusetts General Hospital is one of the country's leading clinical and research hospitals, and houses the nation's largest dermatology department.

14. Describe the qualifications of the key personnel as appropriate to the project. If more space is needed, you may attach resumes no longer than two pages.

The co-investigator and clinical study PI of the project, R. Rox Anderson, MD is director of research of the Wellman Laboratories of Photomedicine and is a world wide recognized expert for laser assisted medical procedures. He developed various new laser procedures and the principle of "Selective Photothermolysis". Dr. Anderson directs the Laser Center in our department, and according to institutional policy must be the PI on the human studies component.

Dieter Manstein, MD has a two year training in dermatology and has performed more than about 100 resurfacing procedures. He obtained also a Masters in physics and performed his diploma thesis "193nm Excimer-laser ablation of the cornea" under supervision of Dr. Franz Hillenkamp at the Institute of Medical Physics and Biophysics of the University of Muenster, Germany with the best possible grade. Since 1998 he is research fellow at the Wellman Laboratory of Photomedicine under supervision of Dr. R. Rox Anderson. One of his four presentations at the 21st annual meeting Am Soc Las Surg Med, New Orleans, 2001 was awarded as the best basic science poster. XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX XXXXXXX. He is the principle investigator and applicant of this proposal. Dr. Manstein recently completed ECFMG certification and is eligible for limited medical licensure, such that he can materially participate in clinical trials.

15. Summarize previous grants on this project received and administered during the past five years.

EXHIBIT B

The concept of 'fractional resurfacing' is new and no previous applications for other grants were submitted for this project.

FUNDING SUPPORT:

16. List other sources of support contacted and indicate their response.

No other sources were contacted yet for funding because this is a new project and we consider the ASMLS as the most appropriate funding source.

17. If this request for funding is denied, what alternative plan do you have for obtaining support?

If this request is denied we will apply for NIH funding.

18. What resources will be available for continuation of this project, if desired?

If this project can demonstrate promising results for resurfacing procedures and further investigation is necessary after termination of this grant we will seek for industrial funding.

19. Is there any additional information you consider helpful to the grant committee in making a decision regarding a grant to your organization?

We consider our laboratory as appropriate to develop and investigate the 'fractional resurfacing' procedure. The personnel are experienced in the development of new laser procedures. The laboratory has the appropriate equipment and human resources for performing and evaluating the in-vitro study, the animal study and the clinical trial. Also confocal microscopy is available within our laboratory. This technique we consider as appropriate to monitor non-invasively the repigmentation and reepithelization process after resurfacing. Preliminary clinical testing at one test site demonstrated that fractional resurfacing enhanced the wound healing process. We are highly motivated to investigate systematically the effects of fractional resurfacing according to the proposed research project.

EXHIBIT B

PROJECT BUDGET:

EXPENSE SUMMARY:

Personnel	
40% Salary of Dr. Manstein	$18.400
30% Research fellow	$10.000
10% Clinical Study Coordinator	$ 3.000
10% Technician support	$ 3.000
Equipment/ Supplies	$ 5.000
Meeting / Travel	$ 1.500
Printing/ Promotion	$ 1.000
Other	
human subjects (10 x $500)	$ 5.000
IRB fee	$ 500
Total project expenses:	**$47.400**

Revenue Summary:

Amount requested from ASMLS:	**$47.400**
Total project revenue:	**$47.400**

Documents to be submitted in support of this application:
- Resumes of key personnel
- List of scientific advisory board
- Organizational chart
- Proof of non-profit status
- Organization brochures

EXHIBIT C